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CHRONIC DISABLING DERMATOSES

ANNUAL PROGRESS REPORT

by

Albert M. Kligman, M. D.

and

Richard R. Marples, B. M.

September 1970

(For the period 1 June 1969 to 31 May 1970)

Supported by

U. S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND
Office of the Surgeon General, Washington, D.C. 20314
in cooperation with the Commission on Cutaneous Diseases
of the Armed Forces Epidemiological Board

Contract No. DA-49-193-MD-2137
University of Pennsylvania
Philadelphia, Pennsylvania 19104

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SUMMARY

Free fatty acids (FFA) liberated from sebum have been implicated in the pathogenesis of acne. To determine the lipase producing organisms three studies were performed. In the first, the scalp was treated topically with neomycin to eliminate aerobic cocci. No change in the percentage of free fatty acids occurred. Next, subjects received oral demethylchlortetracycline to abolish C. acnes. Those subjects with high FFA responded with a 50% reduction. The third study used topical amphotericin B to reduce yeasts (Pityrosporum) and the values for FFA are now being determined.

Quantitative bacteriology and Baird Parker classification of the cocci in acne comedones showed the numerical preponderance of C. acnes. The cocci were almost always SII.

Athletes' foot has been examined with reference to the total microflora. A method for sampling the 4th interspace has been tested. Bacteria play a considerable role in the clinical appearance and course of this basically fungal condition.

Contents and Illustrations

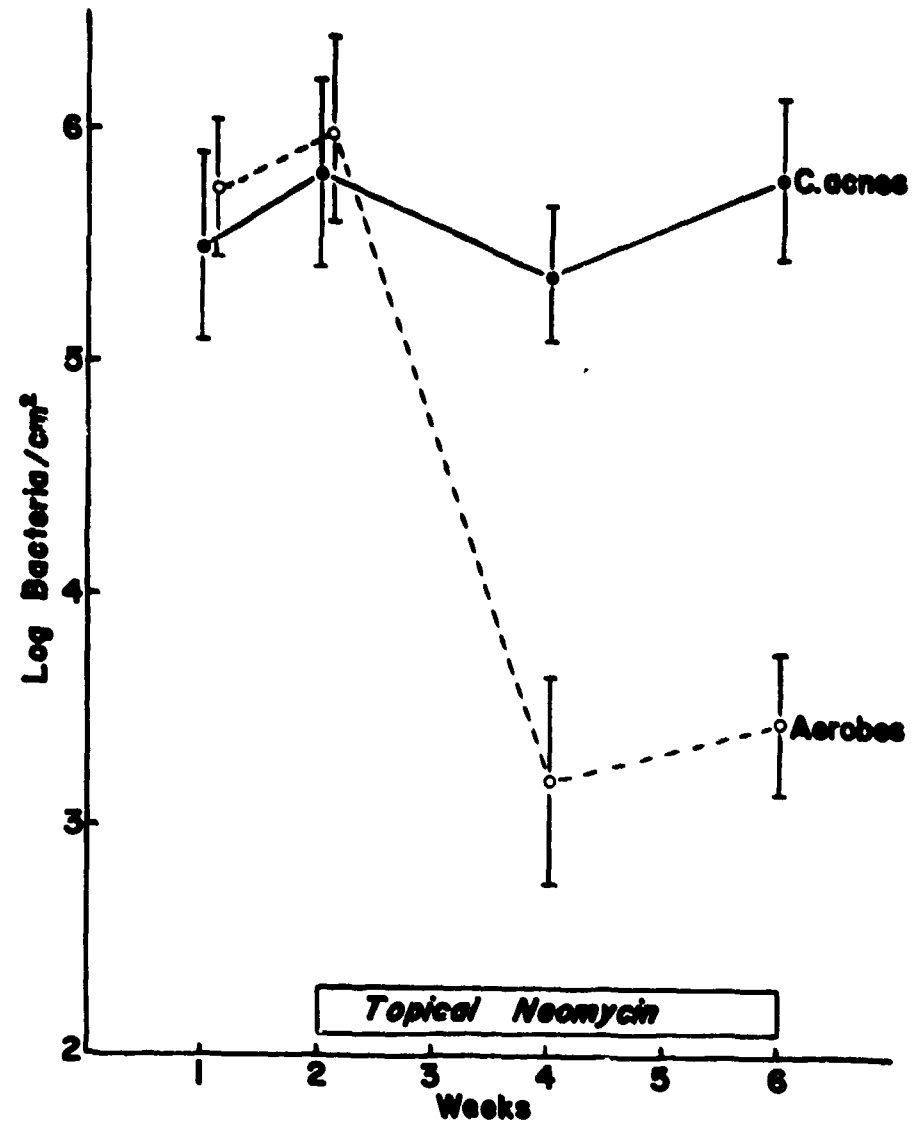
INTRODUCTION

The studies covered by this report relate to two chronic diseases, acne vulgaris and athletes foot, in which the role of bacteria in prolonging and intensifying the disease is poorly understood. Knowledge of the details of bacterial activity and ecology will permit more rational treatments to be developed.

Acne vulgaris and acne tropicalis are substantial sources of disability particularly in tropical areas. The bacterium most often implicated in the pathogenesis of acne is the anaerobic diptheroid Corynebacterum acnes. Because this organism is also found on normal skin it is difficult to establish its pathogenicity.

Athletes foot is usually considered a fungus infection, but significant clinical changes are produced by bacterial secondary infection. The bacterial flora is complex and ecological interactions are a major factor in controlling the ability of pathogens to flourish. Antifungal treatment must not lead to a bacterial infection through ecological changes.

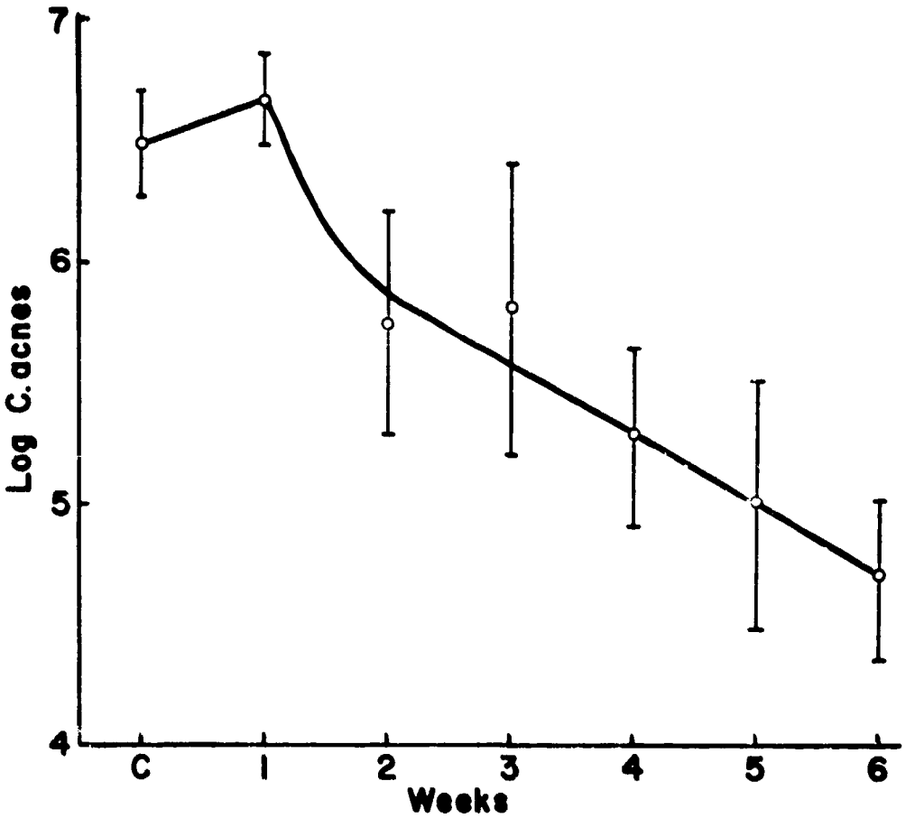
Figure 1



Studies of free fatty acid production:

A major effort over the past 18 months has been to determine the importance of the different microbial species in the production of free fatty acids in the surface lipids. These arise from the hydrolysis of triglycerides contained in sebum. They are worthy of attention because they are implicated in the elicitation of comedones and the creation of inflammatory acne lesions owing to their irritancy. They are also thought to play a role in preventing the colonization of the skin by virulent microbes. The scalp was chosen for these studies since it is easy to sample, the microbial flora is composed of approximately equal numbers of the anaerobic diphtheroid Corynebacterium acnes, coagulase negative cocci (principally M 3 but with substantial numbers of SII in the Baird Parker classification) and lipophilic yeasts of the genus Pityrosporum. Each of these groups has been shown to be able to liberate free fatty acids from triglycerides in the test tube but not in vivo.

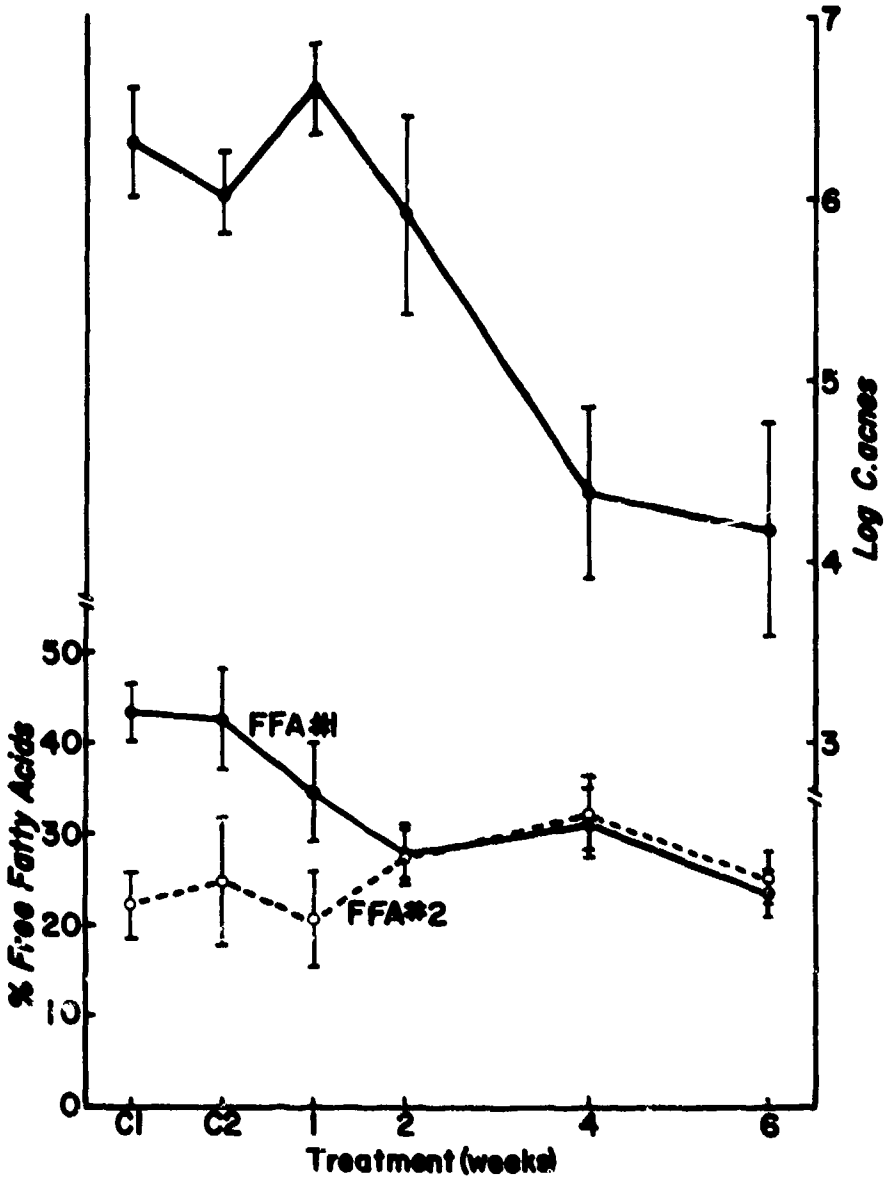
Figure 2



The first study was mentioned briefly in last year's report. Topical aqueous neomycin treatment was used to reduce the coagulase negative cocci. These are situated mainly on the surface and not in the follicles. Aerobic cocci were reduced one hundred fold (Fig. 1) without change in the density of C. acnes. The lipophilic yeasts increased somewhat. No change occurred in the composition of surface lipid. We concluded that aerobic cocci contributed little to the production of free fatty acids.

The second experiment involved administering conventional oral doses of demethylchlortetracycline (DMCT). This antibiotic is delivered slowly to the surface by holocrine secretion, that is, by becoming incorporated in sebaceous gland and epidermal cells which exfoliate. We found previously (Fig. 2) that the density of cocci was not affected by DMCT owing to the emergence of resistant strains but C. acnes declined steadily during 6 weeks of treatment. Pityrosporum yeasts were unaffected. In the current study we gave 9 subjects 600 mgm. of DMCT daily for 6 weeks and followed the changes in: a.) composition of the surface lipids by thin layer chromatography, b.) density of C. acnes c.) density of aerobes d.) numbers of Pityrosporum and e.) antibiotic resistance. The chromatographic studies were carried out by Downing and Strauss in Boston.

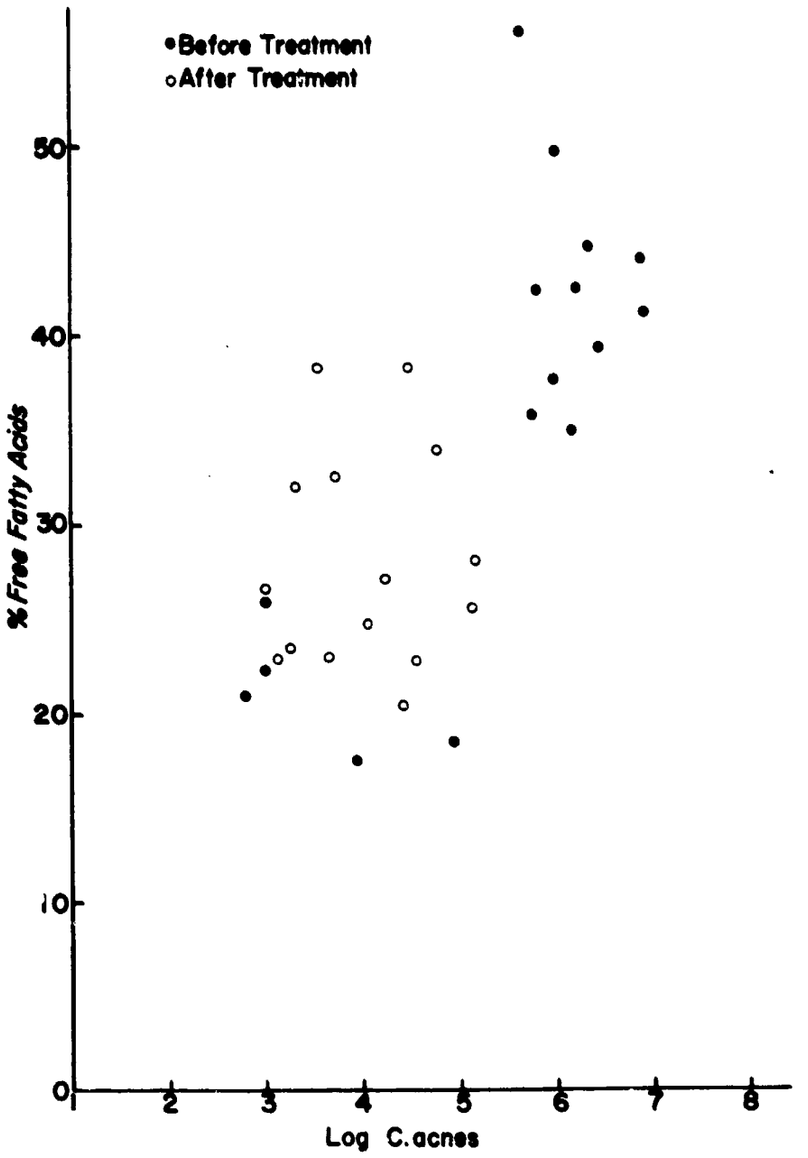
Figure 3



Initially three subjects had unusually low numbers of C. acnes and correspondingly low free fatty acids. In these treatment had no effect. The remaining subjects showed a slow but steady fall in C. acnes and a more rapid decrease in the percentage of free fatty acids (Fig. 3 and Fig. 4.)

These results make it virtually certain that the principle source of triglyceride-splitting lipase is the anaerobic diphtheroid, C. acnes. The level of free fatty acids was strongly correlated with the numbers of C. acnes before treatment and suppression of this organism by DMCT lowered the free fatty acids by almost 50%. However, the decrease in free fatty acid occurred before any change in C. acnes density. This is probably due to the bacterostatic nature of the drug but may also be a result of direct enzyme inhibition. Tetracyclines have been shown to inhibit lipases at levels which permit the growth of C. acnes. Even after DMCT, free fatty acids still constitute a significant percentage of the surface lipids about 20% in our samples.

Figure 4



Studies were done to determine whether Pityrosporum lipases might not be responsible for the failure of DMCT to lower the FFA by more than 50%. Topical 0.5% amphotericin B in carbopol gel was found to reduce the yeast population strongly. The medication was applied daily for four weeks. The results are just now being assembled but it is clear that the aerobic flora and the anaerobic flora did not decrease in density while Pityrosporum was effectively abolished. It remains to be seen from chromatography whether this was accompanied by a lowering of the free fatty acids.

These studies brought to light that certain individuals have unusually low numbers of C. acnes and correspondingly low proportions of free fatty acids. Downing and Strauss in Boston had come across subjects with practically no free fatty acids in their surface lipids. We undertook to study these individuals bacteriologically returning to Philadelphia the same day to set up the cultures. This proved perfectly feasible and points the way to collaborative efforts between widely separated investigators.

It was found that individuals with very low free fatty acids carry an extremely low microbial flora on the forehead. This peculiarity should be investigated since low bacterial carriers may be less vulnerable to intercurrent skin infection. This model might provide some insight into why some individuals are much more susceptible than others to bacterial infection.

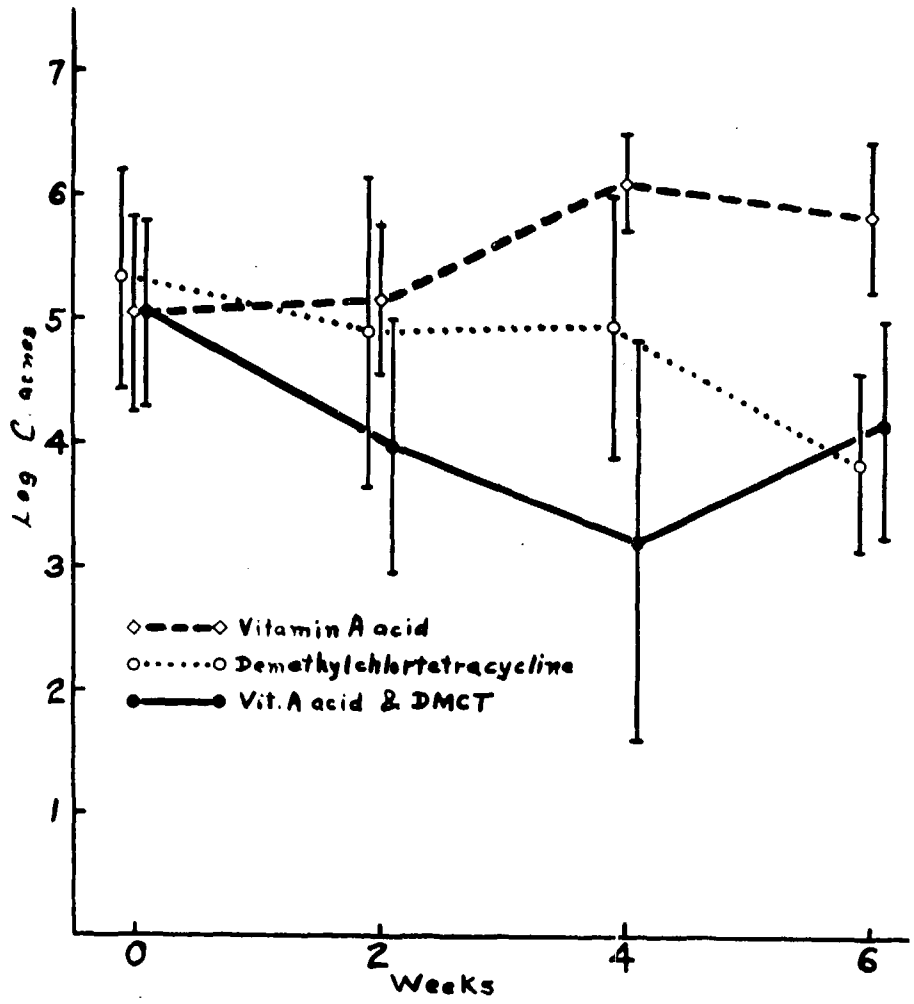
Bacteriology of comedones:

Two organisms, C. acnes and coagulase negative cocci have been consistently isolated from all types of acne lesions. We examined comedones using gravimetric quantification of the density of the two groups and classified the cocci by the Baird Parker system.

C. acnes and coagulase negative cocci were present in every comedo. The geometric mean coccal density was 80,000 organisms per milligram in open comedones and 150,000 in closed comedones. The values for C. acnes were 248,000 and 657,000 organisms per milligram. Statistically this difference is significant as is the great excess of C. acnes over aerobes.

As in acne pustules the cocci were almost always SII. Of the 64 strains tested 54 were SII and 5 were SV. No other group was isolated more than twice.

Figure 5



Acne therapy

Our report on the efficacy of vitamin A acid in acne has led to confirmatory studies. It was natural to inquire whether the combination of tetracycline and retinoic acid would be more effective than either one alone. Some therapeutic gain would be expected on theoretical grounds since it is known that retinoic acid produces an inflammatory response and that DMCT preferentially localizes in areas of inflammation. Three groups of acne patients were compared. One received DMCT, another topical retinoic acid and the third both agents. The subjects were followed with regard to: a.) density of C. acnes, b.) clinical responses (lesion counting) and c.) free fatty acids in the surface lipids.

We concluded that the combination was decidedly more effective than either agent alone. This was particularly true of the more severe cases. In our opinion this is an important advance in the therapy of acne. We plan to evaluate this regimen in acne conglobata with an eye toward tropical acne. We could also show that DMCT was reaching the target more effectively in that the numbers of C. acnes began to fall more rapidly (Fig. 5). It is interesting that the anaerobic count tended to increase with retinoic acid alone.

Studies on athletes' foot:

Athletes' foot is a common disorder which in the civilian population is mainly distressing rather than disabling. In military operations it may become quite severe. It has long been recognized that dermatophytic fungi are important in etiology. However, these are not demonstrable in all cases and other organisms may be variably contributing to the lesion. Our approach has been to examine all the microbial components and to try to relate the symptomatology to the kinds and quantities of organisms present. It is already clear that bacteria are often significant.

This is a new program which will require new techniques and experience. Our present concerns are:

1.) Development of quantitative procedures for assessing the number of yeasts and bacteria.

2.) Qualitative analysis of the composition of the flora. A wide variety of organisms are present.

3.) Response to antifungal and antibacterial agents including vehicles.

4.) Ecological alterations following suppression of specialized groups, viz: fungi, yeast, gram positives and gram negatives.

The fourth toeweb is the most severely and constantly affected site in athletes' foot. Sampling of this space has great limitations but a standardized technique using detergent solutions can give reasonably reliable data on bacterial densities. The technique we presently use is as follows:

1.) 1 ml of sterile Triton X-100 in pH 7.9 phosphate buffer is placed in a sterile tube.

2.) A sterile cotton is moistened with the detergent solution and the excess wrung out on the side of the tube.

3.) The swab is rubbed firmly with rotation 20 left and right times over the web area of the fourth interspace.

4.) The swab is returned to the remaining detergent and the swabstick broken to allow replugging of the tube. This sample is then kept at 0° C until workup.

5.) A second one ml of Triton X-100 solution is added and the tube plus swab firmly agitated using a mechanical mixer to disperse organisms from the swab.

6.) The swab is discarded.

7.) Several tenfold dilutions are set up using half strength wash fluid.

8.) Single drops from an 0.2 ml bacteriological pipette from each dilution are placed on the following media. Because of the detergent these drops are of the same size and spread sufficiently on the agar surface to give countable colonies.

1.) Trypticase Soy Agar (TSA)

2.) TSA with 0.5% Tween 80 as an oleate source for lipophilic diphtheroids.

3.) MacConkey Agar for enterobacteria

4.) Sabourauds agar with penicillin and streptomycin for all yeasts and fungi.

5.) Phenylethanol agar to inhibit enterobacteria.

Streaks are also made on Marshall & Kelseys agar and blood agar.

KOH mounts and cultures for demonstrating dermatophytes are obtained by scraping the interspace with a spatula immediately after bacterial sampling. Because of the moisture left after the sampling procedure a goodly amount of scale can be readily accumulated. Most of the scales are smeared on to a sterile slide and covered with 10% KOH, the remainder is inoculated into DTM medium.

The DTM plates are incubated at room temperature, the remainder at 37°C. The plates are examined at 18 hours for rapidly growing organisms but not finally assessed until the third day for bacterial counts or until 2 weeks for fungi.

This procedure enables a complete analysis of the aerobic flora. Anaerobic plates can also be run but are usually not informative.

The method has been applied to a series of more than 70 individuals. The results are displayed in Figure 6 where the rows indicate different clinical grades and the columns the groups of bacteria. Each graph is a logarithmic distribution running from 10^0 to 10^8.

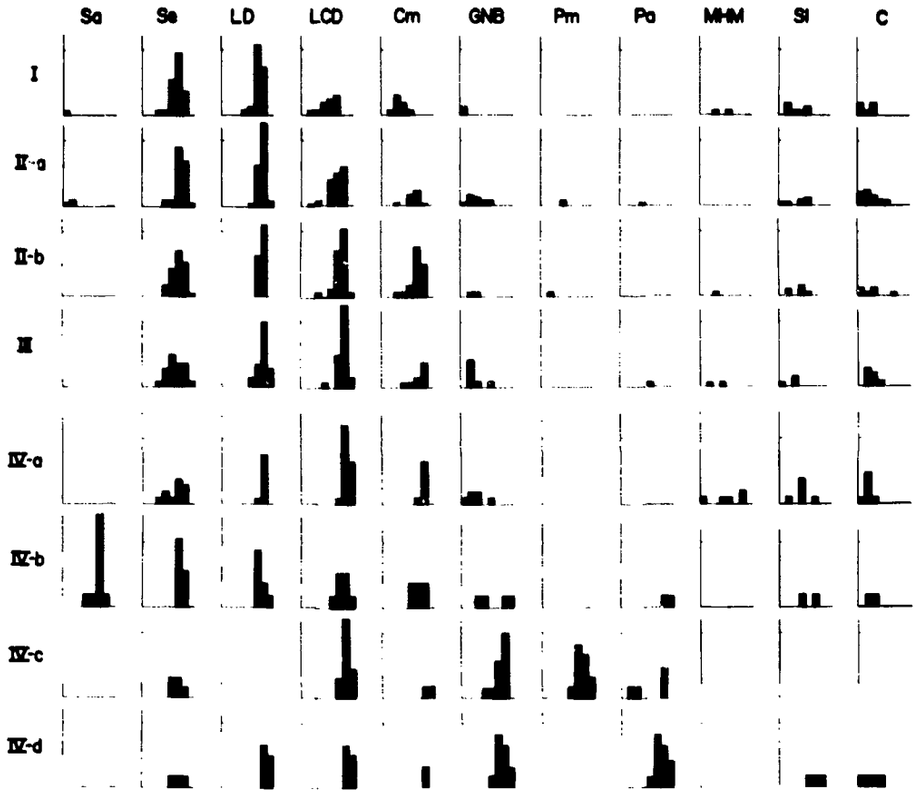
The clinical grades are:

I clinically normal
IIa mild scaling only, KOH positive
IIb mild scaling only, KOH negative
III moderate maceration
IV hyperkeratotic macerated toewebs
  a. without putative pathogen
  b. S. aureus clinically inflamed
  c. Enterobacteria
  d. Significant Pseudomonas aeruginosa

The columns are labelled:

S.a Staphylococcus aureus
S.e Coagulase negative cocci
LD Lipophilic diphtheroids
LCD Miscellaneous diphtheroids
CM Proven C. minutissimum
GNB Gram negative rods
Pm Proteus mirabilis
Pa Pseudomonas aeruginosa
MHM Mima-Herellea-Moraxella group
SI Sarcina lutea
C Candida species

Figure 6



From Figure 6 it is clear that the dominant organisms in the normal and minimally affected toewebs are coagulase negative cocci and particularly lipophilic diphtheroids. While miscellaneous diphtheroids are also present in mild dermatophytosis, the number found to be Corynebacterium minutissimum is markedly higher in those subjects with mild scaling without recovery of a dermatophyte and these subjects must on clinical grounds be called erythrasma although in many instances coral red fluorescence could not be demonstrated.

In the more severe grades dermatophytes were usually recovered. The representation of the normal flora tended to diminish and significant parts of the flora was made up by enterobacteria and Pseudomonas. Those toewebs carrying S. aureus were more inflammatory with a greater tendency for fissure formation.

It seems clear that severe interdigital infections are complex in their microbiology and that treatment must be sufficiently broad or specifically monitored for the best results.

Appendix I

Publications

1.) Marples, R.R.; Fulton, J.E.; Leyden, J. and McGinley, K.J.: Effect of antibiotics on the nasal flora in acne patients. Arch, Derm. 99:647-651, 1969.

2.) Marples, R.R. and Kligman, A.M.: Pyoderma due to resistant Staphylococcus aureus following topical application of neomycin. J. Invest. Derm. 53:11-13, 1969.

3.) McGinley, K.J.; Marples, R.R. and Plewig, G.: A method for visualizing and quantitating the desquamating portion of the human stratum corneum. J. Invest. Derm. 53: 107-111, 1969.

4.) Marples, R.R. and Williamson, P.: Effects of systemic demethylchortetracycline on human cutaneous microflora. Applied Micro. 18:228-234, 1969.

5.) Willis, I. and Kligman, A.M.: Evaluation of sunscreens by human assay. J. Soc. Cosm. Chem. 20:639-651, 1969.

6.) Marples, R.R.: Violagabriellae variant of Staphylococcus epidermidis on normal human skin. J. Bact. 100:47-50, 1969

7.) Willis, I. and Kligman, A.M.: Photocontact allergic reactions. Arch. Derm. 100:535-539, 1969.

8.) Kligman, A.M.: Evaluation of cosmetics for irritancy. Toxicology and Applied Pharm. Supp 3:30-44, 1969.

9.) Marples, R.R. and Izumi, A.K.: Bacteriology of pustular acne. J. Invest. Derm. 54:252-255, 1970.

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13. ABSTRACT

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Corynebacterium acnes						
Pityrosporum						
baird Parker classification						
Athletes' foot						
Staphylococcus aureus						
Proteus mirabilis						
Pseudomonas aeruginosa						
Corynebacterium minutissimum						

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